DOI: 10.1111/j.1570-7458.2008.00753.x

Mortality of *Rhagoletis indifferens* exposed to hydrolyzed protein baits and spinosad in the absence and presence of yeast extract

Wee L. Yee*

United States Department of Agriculture, Agricultural Research Service, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951, USA

Accepted: 11 June 2008

Key words: western cherry fruit fly, Diptera, Tephritidae, GF-120, Nu-Lure, Mazoferm, sugar

Abstract

Western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), is the major quarantine pest of sweet cherry, *Prunus avium* (L.) L. (Rosaceae), in the Pacific northwest of the USA and in British Columbia in Canada. Although spinosad bait (GF-120 NF Naturalyte® Fruit Fly Bait) is used for the control of *R. indifferens* in this region, the effects of alternate food sources on fly responses to this bait have not been studied. In this study, experiments were conducted to determine mortalities of flies exposed to hydrolyzed protein baits in the presence of sugar only and sugar + yeast extract food. All baits contained Entrust® (termed 'spinosad alone'). When flies were exposed to GF-120 with or without added ammonia compounds (uric acid, ammonium acetate, and ammonium carbonate) for 48 h, mortalities were higher in the presence of sugar only than in the presence of sugar + yeast extract, but when flies were exposed to spinosad alone, mortalities were similar in presence of either of the two foods. In another experiment comparing GF-120, Nu-Lure, Mazoferm, Baker's yeast extract, and spinosad alone, mortalities in the GF-120, Mazoferm, and Baker's yeast extract treatments were higher in the presence of sugar only than in the presence of sugar + yeast extract, but in the Nu-Lure and spinosad alone treatments, mortalities were similar in the presence of either of the two foods. Overall results suggest that the indirect effects of yeast extract food on mortality are dependent on bait type and that mortalities caused by spinosad alone and baits are similar. Nu-Lure and spinosad alone may have an advantage over other treatments for fly control, because their effects do not appear to be affected by the presence of nitrogenous food.

Introduction

Western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), is the major pest of sweet cherries, *Prunus avium* (L.) L. (Rosaceae), in the Pacific northwest of the USA (Washington state and Oregon) and in British Columbia in Canada. There is a zero tolerance for cherry fruit fly larvae in commercial fruit (State of Washington Department of Agriculture, Permanent Order No. 1099, effective 30 September 1968), so insecticides continue to be vital in managing the fly. Spinosad bait (GF-120 NF Naturalyte® Fruit Fly Bait; Dow AgroSciences, Indianapolis, IN, USA), composed of spinosad (0.02%), 1% ammonium acetate (AA), hydrolyzed maize protein (solulys), and other ingredients (listed in Thomas & Mangan, 2005), is used for fly control in Washington State. It is effective in causing high mortality in or managing *R. indifferens* (Yee & Chapman, 2005) and other tephritids (Prokopy et al., 2003; McQuate et al., 2005; Mangan et al., 2006). Spinosad is an insecticide derived from fermentation products of the soil bacterium *Saccharopolyspora spinosa* Mertz and Yao that can also be used alone for managing fruit flies (Pelz et al., 2005) and other insects (De Deken et al., 2004; Huang & Subramanyam, 2007).

The abundance of alternate food sources in the environment may have an effect on the responses of flies to hydrolyzed protein baits, such as GF-120. In nature, trees may have low- or high-nitrogen abundance throughout the season, perhaps in bacteria, which appear to be a natural food for some fruit flies (Drew et al., 1983) and bird feces (Yee, 2008), such that a fly can feed on little or much nitrogenous food.

*Correspondence: E-mail: wee.yee@ars.usda.gov

The protocol for testing baits for tephritids usually is to feed flies nitrogenous or sugar food before testing, after which flies are no longer exposed to the food. In general, flies deprived of nitrogenous food respond more to baits than those that had access to it (Prokopy et al., 1992; Vargas et al., 2002). Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), that were continuously exposed to nitrogenous food were less attracted to bird feces and yeast hydrolysate than flies deprived of it (Prokopy et al., 1992). In the apple maggot, *Rhagoletis pomonella* (Walsh), bird feces possibly can compete with protein bait sprays (Prokopy et al., 1993). In *R. indifferens*, exposure to yeast extract prior to exposure to baits reduced the numbers of feeding events on them (Yee, 2006).

Despite the use of GF-120 for fly control, it is not clear if bait is a necessary component for spinosad to be effective. GF-120 did not appear attractive to *R. indifferens*, unless flies were starved (Yee & Chapman, 2005), and adding additional AA to it was needed to make it attractive for the eastern cherry fruit fly, *Rhagoletis cingulata* (Loew) (Pelz-Stelinski et al., 2006). Adding 10% AA to it enhanced its efficacy in reducing larval infestations of *R. indifferens* in fruit, depending on how infestations were measured (Alston & Lindstrom, 2006). Spinosad alone also is not attractive and did not differ from GF-120 in controlling flies, although there was much variability within treatments (Yee, 2007a), and it would be expected that visual cues alone would draw more flies to GF-120 than to spinosad alone. Whether different types of hydrolyzed protein baits differ in effectiveness and whether the presence of nitrogenous food differentially affects results using these baits and spinosad need to be studied.

In this study, the major objectives were to determine the mortality of *R. indifferens* exposed to various hydrolyzed protein baits in the absence and presence of sugar + yeast extract food, a source of amino acids and nitrogen. Three related hypotheses were tested: (1) the absence of yeast extract results in higher mortality of flies exposed to bait and spinosad than the presence of it; (2) fly mortality caused by spinosad alone is less than caused by baits with spinosad; and (3) fly mortality is dependent on the type of bait. A minor objective was to determine the behavioral responses of flies to baits, to provide clues to a mechanism of kill that may explain mortality patterns.

Materials and methods

Flies and fly maintenance before experiments

Flies originated as larvae reared from sweet cherries in Richland and Kennewick, WA, USA, in June and July 2005 and 2006. Puparia were stored inside containers in moist peat moss, sand, and vermiculite at 3 °C or at 3 °C and then 11 °C for at least 6 months. Puparia were then transferred to 27 °C for adult emergence. Prior to all three experiments below, flies were maintained only on 5% sucrose (wt/wt) in cotton wicks and water from emergence. A maximum of 50 males and 50 females was held per 1.9-l (10.5 cm high × 16.2 cm diameter) paper container. The same type of container was used in the three experiments below. Flies in all experiments were tested at 6 or 7 days post-emergence. All experiments were conducted at 26–27 °C, 30–40% r.h., and under an L16:D8 photoperiod with light intensity of ca. 4800 lumen/m^2.

Experiment 1: effects of sugar only and sugar + yeast extract, enhanced GF-120, and exposure time on mortality

In this experiment, there was a sugar only and a sugar + yeast extract food treatment for a control and five bait treatments. Each food was presented dried on a 5 × 9 cm strip of unbleached paper towel. For the sugar only food, paper strips were soaked in 50% sucrose (wt/wt) (C & H Sugar, Crockett, CA, USA) and air dried. For the sugar + yeast extract food, paper strips were dipped in a solution of 12.5% yeast extract (yeast extract granulated: EMD Chemicals, Gibbstown, NJ, USA) and 50% sucrose (wt/wt) and dried to yield a 20% yeast extract–80% sucrose food. The amounts of dried sugar and sugar + yeast extract exposed to flies were ca. 300 and 500 mg (= 400 mg sugar), respectively. Food was unlimited, because in *R. pomonella*, the maximum consumptions of dried sucrose and dried yeast hydrolysate were 0.81 and 0.29 mg/fly/day, respectively (Webster et al., 1979). The control and five bait treatments for each food were: (1) water control (no sugar in water); (2) 20% GF-120 (vol/vol) [undiluted GF-120 has 1% AA or 10 g AA/l pre-mixed (Thomas & Mangan, 2005)]; (3) 20% GF-120 + 10% uric acid (wt/wt) (UA) (108 g UA/l) (99+%; Acros Organics, Geel, Belgium); (4) 20% GF-120 + 10% AA (107 g AA/l) (97+%; Aldron, Avon, NY, USA) (in addition to 1% AA already present); (5) 20% GF-120 + 10% ammonium carbonate (AC) (109 g AC/l) (Keystone Universal, Melvindale, MI, USA); and (6) spinosad alone (Entrust®; Dow AgroSciences). Entrust® is 80% spinosad (spinosyns A and D) (wt/wt) and 20% inert proprietary ingredients, but for convenience in this article, Entrust® is referred to as 'spinosad alone'. Densities of GF-120 baits ranged from 1.03 to 1.08 g/ml. All GF-120 treatments contained 0.05 g spinosad/l, as did spinosad alone. Three 50-µl drops of water, baits, or spinosad alone were applied using a pipette (LTS 200 pipette with a 250-µl LST tip) (Rainin Instrument, Oakland, CA, USA) onto a clear Petri dish (1.3 cm high × 9.0 cm diameter) and allowed to dry for 1 h at 20–21 °C. The dish was then introduced onto the bottom of a 1.9-l container with 15 male and 15 female flies that had been placed in it

ca. 30 min before. Each container had two water wicks plugged in 12-ml glass vials and one food strip held with a paper clip onto one side, 7–8 cm from the bait drops. Fly mortalities were recorded at 2, 4, 6, 8, 24, 26, 28, 30, and 48 h after exposure to treatments. Numbers of dead flies (sexed) were counted. Flies that were paralyzed and could not walk or that were motionless were counted as dead. Dead flies were not removed during the 48 h. There were five replicates of the control and treatments, except in the sugar + yeast extract and spinosad alone treatment, where there were four replicates.

Experiment 2: effects of sugar only and sugar + yeast extract, hydrolyzed protein baits, and exposure time on mortality

This experiment was similar to experiment 1, except GF-120 and other hydrolyzed protein baits were tested and flies were exposed to 10 50-µl drops of water, baits, or spinosad alone on a Petri dish. The higher drop numbers may affect how quickly and how many flies encounter the drops. For the sugar only and sugar + yeast extract food treatments, there was a control and five bait treatments. Treatments were: (1) water control (no sugar in water); (2) 20% blank GF-120 (without pre-mixed spinosad); (3) 20% Nu-Lure (Scentry Biologicals, Billings, MT, USA); (4) 20% Mazoferm (Corn Products, Bedford Park, IL, USA); (5) 20% yeast extract (EMD Chemicals, Gibbstown, NJ, USA); to avoid confusion with yeast extract used as food, this treatment is referred to as 'Baker's YE', because it was Baker's yeast extract; and (6) spinosad alone. Densities of the hydrolyzed protein baits ranged from 1.02 to 1.08 g/ml. The approximate percentage sugars (wt/vol) in the 20% concentration of GF-120, Nu-Lure, Mazoferm, and Baker's YE were 4, 4, 0.1–0.4, and 0%, respectively. A rate of 0.05 g spinosad in Entrust®/l was added to all bait treatments except the water control. Spinosad alone (Entrust®) also was 0.05 g spinosad/l. Numbers of flies per container and methods for recording mortality were the same as in experiment 1. There were five replicates of the control and each treatment.

To confirm that pre-packaged GF-120, which has 0.02% spinosad (wt/vol) pre-mixed and not added as Entrust®, has the same effect as blank GF-120 + Entrust®, another test was conducted comparing this bait in the presence of sugar only and sugar + yeast extract. Methods were the same as in the previous paragraph. There were five replicates of 30 flies for each food treatment.

An additional test comparing spinosad alone in the presence of sugar only and sugar + yeast extract was conducted. The methods were the same as described above for spinosad alone, except that 0.09 g spinosad/l instead of 0.05 g/l was tested. There were five replicates of 30 flies for each food treatment.

Experiment 3: effects of enhanced GF-120 on feeding responses

The objective here was to determine the behavioral responses of flies to enhanced GF-120 drops that may explain mortality patterns in experiment 1 (above). Five male and five female flies were placed inside a 1.9-l container with a 55-cm^2 green silk leaf (Silk Garden Shop, Michaels Store, Irving, TX, USA) with six fresh 25-µl drops of the same treatments as in experiment 1. Drops were applied 5 min before flies were introduced into containers. A leaf was used instead of a Petri dish in order to obtain data more quickly, because initial observations suggested that flies used the leaves as resting substrates more than dishes. The leaf, glued onto a clear plastic vial (5.5 cm high), was parallel to the bottom of the container. There was a space of 5 cm between the leaf and top of the container. A clear acetate sheet was placed on half of the top of each container, so flies could be viewed easily. The other half was covered with light bridal cloth for ventilation. Data recorded were numbers of feeding events over 60 min, durations of feeding events, and durations spent by flies on the leaves without feeding over this time. There were no water vials in containers during observations. There were six replicates of the control and each treatment.

Statistical analysis

Three types of analysis of variance (ANOVA) were conducted: (1) repeated-measures, (2) two-way, and (3) one-way. (1) For experiments 1 and 2, three-way repeated-measures ANOVA (Littell et al., 1996) was conducted on fly mortality (%). Fixed factors were food and bait [the two between-subjects (categorical) factors] and time after exposure to bait [the one within-subjects (quantitative) factor], with repeated measures (mortalities) on the time factor, as mortalities were recorded on flies within the same replicate cages over time. Repeated-measures ANOVA was also conducted within a bait treatment, using food treatment and time after exposure to bait treatment as factors. (2) For experiments 1 and 2, two-way ANOVA was conducted on mortality data at 2, 8, 24, and 48 h after exposure, using food and bait as the two factors. (3) One-way ANOVA of mortality within bait treatments in sugar only vs. sugar + yeast extract food groups at 2, 8, 24, and 48 h after exposure was also conducted. For the test using GF-120 pre-mixed with spinosad and test using 0.09 g spinosad/1 alone and food types, repeated-measures ANOVA was conducted. Percent mortality was arcsine $\sqrt{x}$ transformed prior to analyses. For experiment 3, one-way ANOVA was conducted for numbers of feeding events $(y + 0.5)$ and durations spent on leaves without feeding. Fisher's least significant difference test was used to separate means after ANOVA in all experiments. In experiment 3, water,

GF-120 + AC, and spinosad alone treatments had only one or two replicates with feeders, so feeding durations for these were not analyzed. The Proc MIXED and Proc GLM procedures in SAS (SAS Institute, 2004) were used for analyses.

Results

Experiment 1: effects of sugar only and sugar + yeast extract, enhanced GF-120, and exposure time on mortality

Female and male mortality data were combined, because there was no evidence of sex differences, for example, mortality 24 h after exposure to GF-120: 36.7 and 48.3% mortality in females (n = 30) and males (n = 29), respectively (Fisher's exact test: P = 0.43). Mortalities of flies exposed to water (Figure 1A) in the presence of sugar only and sugar + yeast extract were similar, but mortalities of flies exposed to GF-120 (Figure 1B), GF-120 + UA (Figure 1C), GF-120 + AA (Figure 1D), and GF-120 + AC (Figure 1E) were higher in the presence of sugar only than in the presence of sugar + yeast extract. Mortalities of flies exposed to spinosad alone (Figure 1F) in the presence of the two foods did not differ at any time after exposure.

Repeated-measures ANOVA (Table 1) indicated that there were significant food, bait, and time effects. There were no food*time, food*bait, and food*bait*time interactions. However, there was a significant bait*time interaction. This indicated that the patterns of mortality over time differed among baits. Analyses of individual baits (Table 1) resulted in a food*time interaction only in the GF-120 + AC treatment. There were food effects in the GF-120 and GF-120 + AC treatments and time effects for all treatments.

Two-way ANOVA (Table 2) showed that mortality in bait treatments (hereafter, 'treatments') at 2 h was higher in presence of sugar only than in presence of sugar + yeast extract, that mortalities caused by spinosad alone were

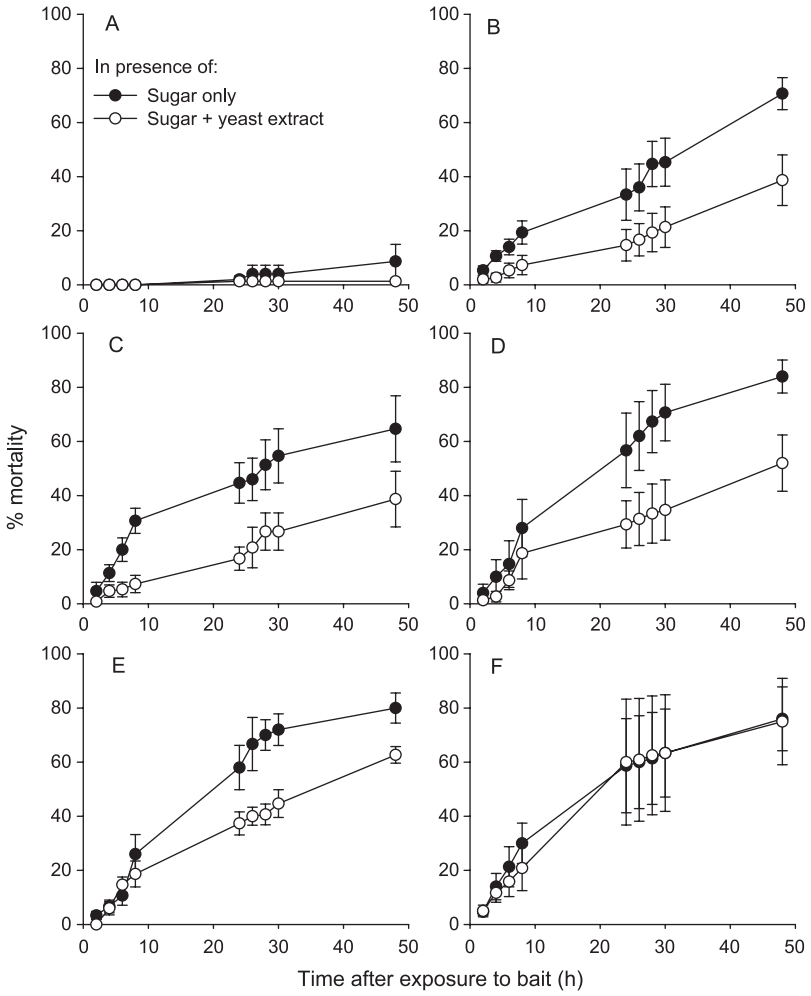

Figure 1 Experiment 1: mortality (mean ± SE) of *Rhagoletis indifferens* exposed to enhanced GF-120 in the presence of sugar only and sugar + yeast extract at 2–48 h after exposure: three drops of 50 µl of (A) water, (B) GF-120, (C) GF-120 + 10% uric acid (UA), (D) GF-120 + 10% ammonium acetate (AA), (E) GF-120 + 10% ammonium carbonate (AC), and (F) spinosad alone. All treatments except water contained 0.0048% spinosad.

Table 1 Repeated-measures analysis of variance (ANOVA) results for the effects of food, GF-120 baits, and time after exposure on mortality of *Rhagoletis indifferens*

Factor	d.f.	F	P
Food	1,45	10.4	0.0024
Bait	5,45	13.8	<0.0001
Time after exposure	8,357	76.5	<0.0001
Food*time	8,357	1.7	0.0942
Bait*time	40,357	4.4	<0.0001
Food*bait	5,45	0.8	0.5225
Food*bait*time	40,357	1.1	0.3520

Repeated-measures ANOVA of individual baits

	Water			GF-120			GF-120 + UA		
Factor	d.f.	F	P	d.f.	F	P	d.f.	F	P
Food	1,8	0.2	0.6458	1,8	7.1	0.0282	1,7	2.8	0.1351
Time	8,64	4.1	0.0006	8,64	19.5	<0.0001	8,54	9.2	<0.0001
Food*time	8,64	1.1	0.3911	8,64	0.9	0.4941	8,54	1.3	0.2623
	GF-120 + AA			GF-120 + AC			Spinosad alone		
Factor	d.f.	F	P	d.f.	F	P	d.f.	F	P
Food	1,8	4.0	0.0805	1,7	12.9	0.0088	1,7	0.0	0.9790
Time	8,64	18.5	<0.0001	8,55	48.0	<0.0001	8,56	12.8	<0.0001
Food*time	8,64	2.0	0.0649	8,55	3.7	0.0015	8,56	0.2	0.9825

Five replicates of 30 flies each, except for sugar + yeast extract food and spinosad alone bait (four replicates). UA = 10% uric acid, AA = 10% ammonium acetate, AC = 10% ammonium carbonate.

higher than by GF-120 + AC, and that GF-120, GF-120 + UA, GF-120 + AA, and GF-120 + AC treatments did not differ. At 8 h after exposure, mortality also was higher in the presence of sugar only than in the presence of sugar + yeast extract, and mortality in the spinosad alone treatment was higher than in the GF-120 treatment. There were no differences among GF-120 baits (Table 2). At both 24 and 48 h, mortalities were higher in the presence of sugar only than in the presence of sugar + yeast extract, and they were higher in the spinosad alone than in the GF-120 and GF-120 + UA treatments. Mortality in the GF-120 + AC treatment was higher than in the GF-120 treatment (Table 2).

Results of one-way ANOVA of mortalities within bait treatments in sugar only vs. sugar + yeast extract groups at 2, 8, 24, and 48 h after exposure showed that in all except the water and the spinosad alone treatment ($P>0.05$), mortalities were higher in the presence of sugar only than in the presence of sugar + yeast extract in at least one of the four times after exposure. For GF-120, this occurred at 48 h ($F_{1,8} = 7.7$, $P = 0.0242$); for GF-120 + UA at 8 and 24 h ($F_{1,8} = 12.8$ and 7.7, $P = 0.0071$ and 0.0243, respectively); for GF-120 + AA at 48 h ($F_{1,8} = 6.8$, $P = 0.0316$); and for GF-120 + AC at 2 and 48 h ($F_{1,8} = 5.6$ and 6.6, $P = 0.0448$ and 0.0334, respectively).

Experiment 2: effects of sugar only and sugar + yeast extract, hydrolyzed protein baits, and exposure time on mortality

Mortalities of flies exposed to water in the presence of sugar only and sugar + yeast extract were similar (Figure 2A). Mortalities of flies exposed to GF-120 (Figure 2B) were higher in the presence of sugar only than in the presence of sugar + yeast extract. In contrast, mortalities of flies exposed to Nu-Lure (Figure 2C) were similar in the presence of the two foods. Mortalities of flies exposed to Mazoferm (Figure 2D) were higher in presence of sugar only than in the presence of sugar + yeast extract at 24 to 48 h. Mortalities of flies exposed to Baker's YE (Figure 2E) were higher in the presence of sugar only than in the presence of sugar + yeast extract at 6 to 48 h. Mortalities of flies exposed to spinosad alone (Figure 2F) between the two foods were similar.

Repeated-measures ANOVA (Table 3) indicated that there were significant food, bait, and time effects. There were no food*time, food*bait, and food*bait*time interactions. However, there was a significant bait*time

Table 2 Results of two-way analysis of variance (ANOVA) of mortality of *Rhagoletis indifferens* at 2, 8, 24, and 48 h after exposure to 20% (vol/vol) GF-120 baits in the presence of sugar only and sugar + yeast extract

		Mortality (% ± SE)			
Factor		2 h	8 h	24 h	48 h
Food	Sugar	3.7 ± 0.9a	22.3 ± 3.1a	42.2 ± 5.5a	64.0 ± 5.7a
	S + Y	1.4 ± 0.5b	11.8 ± 2.5b	25.4 ± 4.8b	43.7 ± 5.5b
Bait	Water	0.0 ± 0.0c	0.0 ± 0.0c	1.7 ± 0.8d	5.0 ± 3.2d
	GF-120	3.7 ± 1.2ab	13.3 ± 3.3b	24.0 ± 6.1bc	54.7 ± 7.5bc
	GF-120 + UA[1]	3.0 ± 1.9abc	19.0 ± 4.7ab	30.7 ± 6.2bc	51.7 ± 8.7bc
	GF-120 + AA	2.7 ± 1.6abc	23.3 ± 6.9ab	43.0 ± 8.9ab	68.0 ± 7.8ab
	GF-120 + AC	1.7 ± 0.9bc	22.3 ± 4.3ab	47.7 ± 5.6a	71.3 ± 4.2a
	Spinosad alone	4.8 ± 1.1a	25.9 ± 5.4a	59.3 ± 13.2a	75.5 ± 9.0a
ANOVA of factors	Food	$F_{1,46} = 4.4$, P = 0.0410	$F_{1,47} = 10.0$, P = 0.0028	$F_{1,47} = 5.8$, P = 0.0203	$F_{1,47} = 11.3$, P = 0.0015
	Bait	$F_{5,46} = 2.9$, P = 0.0237	$F_{5,47} = 10.0$, P<0.0001	$F_{5,47} = 10.1$, P<0.0001	$F_{5,47} = 17.9$, P<0.0001
	Food*bait	$F_{5,46} = 0.6$, P = 0.7039	$F_{5,47} = 1.2$, P = 0.3425	$F_{5,47} = 0.7$, P = 0.6125	$F_{5,47} = 0.8$, P = 0.5662

Means within columns followed by the same letter are not significantly different (P>0.05).
Five replicates of 30 flies each, except for sugar + yeast extract food and spinosad alone bait (four replicates). S + Y = sugar + yeast extract, UA = 10% uric acid, AA = 10% ammonium acetate, AC = 10% ammonium carbonate.
[1]One missing observation: 2 h, sugar + yeast extract, GF-120 + UA.

interaction. This indicated that the patterns of mortality over time differed among baits. Within individual baits (Table 3), there were food*time interactions in the GF-120 and Mazoferm treatments. Food effects were seen in GF-120 and Mazoferm treatments and time effects were seen in all treatments.

Two-way ANOVA (Table 4) showed that at 2 h, mortalities in the presence of sugar only and sugar + yeast extract were

Table 3 Repeated-measures analysis of variance (ANOVA) results for the effects of food, various bait treatments, and time after exposure on mortality of *Rhagoletis indifferens*

Factor	d.f.	F	P			
Food	1,48	14.7	0.00004			
Bait	5,48	54.7	<0.0001			
Time after exposure	8,384	202.8	<0.0001			
Food*time	8,384	1.4	0.1959			
Bait*time	40,384	8.0	<0.0001			
Food*bait	5,48	2.0	0.1013			
Food*bait*time	40,384	1.2	0.1864			
	Repeated-measures ANOVA of individual baits					
	Water		GF-120		Nu-Lure	
Factor	F	P	F	P	F	P
Food (d.f. = 1,8)	0.2	0.6769	7.2	0.0282	0.5	0.5038
Time (d.f. = 1,78)	63.2	<0.0001	373.1	<0.0001	316.6	<0.0001
Food*time (d.f. = 1,78)	0.1	0.7766	0.6	0.4560	0.3	0.5730
	Mazoferm		Baker's YE		Spinosad alone	
Factor	F	P	F	P	F	P
Food (d.f. = 1,8)	5.2	0.0516	3.7	0.0904	0.2	0.6369
Time (d.f. = 1,78)	306.8	<0.0001	355.1	<0.0001	635.0	<0.0001
Food*time (d.f. = 1,78)	21.2	<0.0001	6.6	0.0122	3.1	0.0821

Five replicates of 30 flies each.

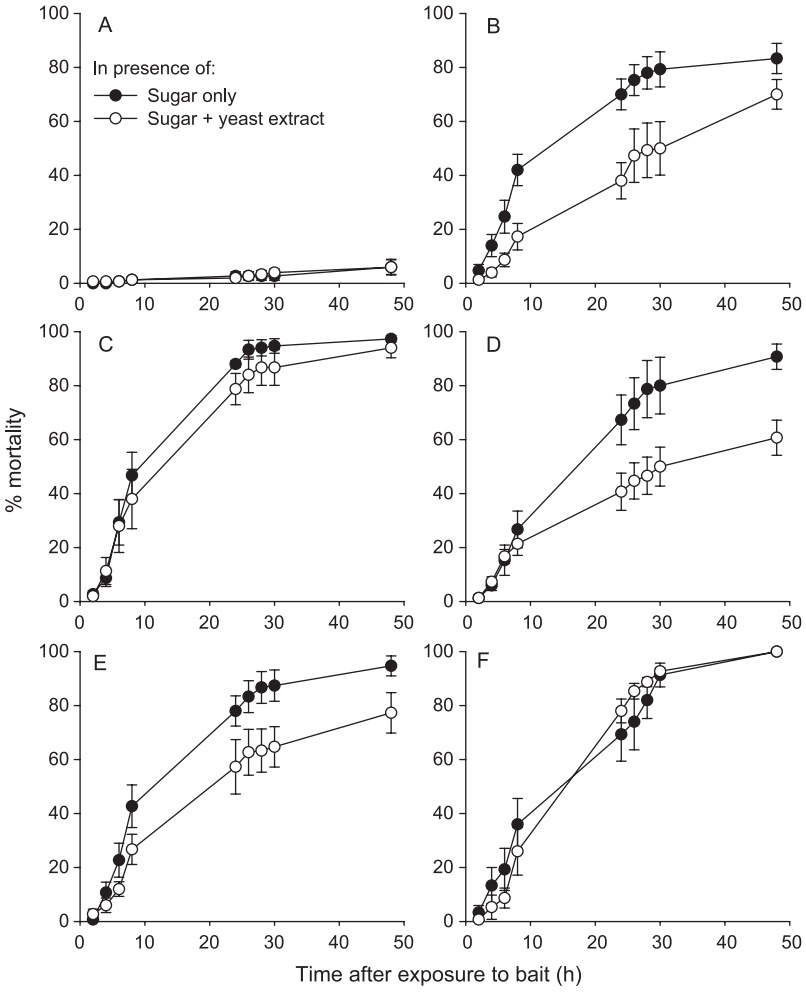

Figure 2 Experiment 2: mortality (mean ± SE) of *Rhagoletis indifferens* exposed to various baits in the presence of sugar only and sugar + yeast extract at 2–48 h after exposure: 10 drops of 50 µl of (A) water control, (B) GF-120, (C) Nu-Lure, (D) Mazoferm, (E) Baker's YE, and (F) spinosad alone. All treatments except water contained 0.0048% spinosad.

similar for all treatments. At 8 h, mortalities were higher in the presence of sugar only than in the presence of sugar + yeast extract. Mortalities were higher in the Nu-Lure than Mazoferm treatment, and mortalities in GF-120, Baker's YE, and spinosad alone treatments did not differ from either (Table 4). At 24 h, mortality was higher in the presence of sugar only than in the presence of sugar + yeast extract, with mortalities higher in the Nu-Lure and spinosad alone than GF-120 and Mazoferm treatments. Mortality in the Baker's YE treatment was lower than in the Nu-Lure treatment (Table 4). At 48 h, patterns of mortality between food and bait factors differed, resulting in a significant food*bait interaction (Table 4).

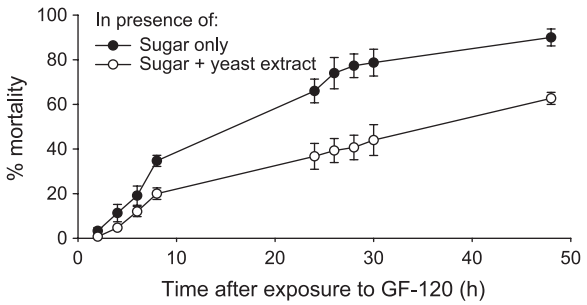

Figure 3 Experiment 2: mortality (mean ± SE) of *Rhagoletis indifferens* exposed to 10 drops of 50 µl of GF-120 (pre-mixed with spinosad) in the presence of sugar only and sugar + yeast extract at 2–48 h after exposure.

One-way ANOVA of sugar only vs. sugar + yeast extract treatments within bait treatments showed that for water, Nu-Lure, and spinosad alone, the presence of sugar only and sugar + yeast extract had no effect on mortalities at 2, 8, 24, and 48 h after exposure. However, for GF-120, Mazoferm, and Baker's YE, the presence of sugar only resulted in higher mortality in at least one of the four times. For GF-120, this occurred at 8 and 24 h ($F_{1,8}$ = 10.0 and 12.5, P = 0.0135 and 0.0077, respectively), for Mazoferm at 48 h ($F_{1,8}$ = 13.8, P = 0.0059), and for Baker's YE at 48 h ($F_{1,8}$ = 6.6, P = 0.0329).

Table 4 Results of two-way analysis of variance (ANOVA) of mortality of *Rhagoletis indifferens* at 2, 8, 24, and 48 h after exposure to various bait [20% (vol/vol)] treatments in the presence of sugar only and sugar + yeast extract

		Mortality (% ± SE)			
Factor		2 h	8 h	24 h	48 h
Food	Sugar	2.1 ± 0.6ns	32.6 ± 3.9a	62.6 ± 5.7a	78.7 ± 6.3[1]
	S + Y	1.4 ± 0.4ns	21.8 ± 3.2b	49.1 ± 5.5b	68.0 ± 6.0[1]
Bait	Water	0.3 ± 0.3ns	1.3 ± 0.5c	2.3 ± 0.9d	6.0 ± 1.8[1]
	GF-120	3.0 ± 1.3ns	29.7 ± 5.5ab	54.0 ± 6.8c	76.7 ± 4.3[1]
	Nu-Lure	2.3 ± 0.7ns	42.3 ± 6.8a	83.3 ± 3.2a	95.7 ± 1.9[1]
	Mazoferm	1.3 ± 0.7ns	24.0 ± 3.9b	54.0 ± 7.0c	75.7 ± 6.2[1]
	Baker's YE	1.7 ± 1.0ns	34.7 ± 5.3ab	67.7 ± 6.4bc	86.0 ± 4.9[1]
	Spinosad alone	2.0 ± 1.3ns	31.0 ± 6.4ab	73.7 ± 5.3ab	100.0 ± 0.0[1]
ANOVA of factors	Food	$F_{1,48} = 0.3$, P = 0.5609	$F_{1,48} = 7.1$, P = 0.0105	$F_{1,48} = 10.9$, P = 0.0018	$F_{1,48} = 15.0$, P = 0.0003
	Bait	$F_{5,48} = 1.1$, P = 0.3621	$F_{5,48} = 14.6$, P<0.0001	$F_{5,48} = 46.0$, P<0.0001	$F_{5,48} = 94.9$, P<0.0001
	Food*bait	$F_{5,48} = 0.7$, P = 0.6088	$F_{5,48} = 0.8$, P = 0.5837	$F_{5,48} = 2.3$, P = 0.0574	$F_{5,48} = 3.2$, P = 0.0143

Five replicates of 30 flies each. S + Y = sugar + yeast extract.
Means within columns followed by the same letter are not significantly different (P>0.05).
ns, not significant.
[1]Means not separated because of the significant food*bait interaction.

In the test of GF-120 with pre-mixed spinosad (Figure 3), results were similar to using blank GF-120 with added spinosad (Entrust®) (Figure 2B), in that mortalities were higher in the presence of sugar only than in the presence of sugar + yeast extract at all times. Repeated-measures ANOVA showed that there were food ($F_{1,8} = 25.8$, P = 0.0010) and time ($F_{8,64} = 49.9$, P<0.0001) effects but no food*time interaction ($F_{8,64} = 1.7$, P = 0.12).

Mortality patterns of flies exposed to 0.0092% spinosad alone (Figure 4) in the presence of sugar only and sugar + yeast extract were similar to those seen with 0.0048% spinosad (Figures 1F and 2F). Repeated-measures ANOVA indicated that there was no food effect ($F_{1,8} = 0.1$, P = 0.72), but there was a time effect ($F_{8,64} = 27.3$, P<0.0001). There was no food*time interaction ($F_{8,64} = 0.4$, P = 0.92).

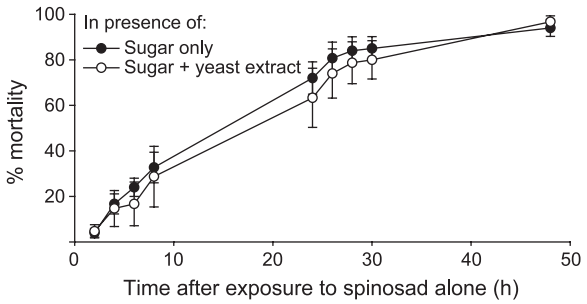

Figure 4 Experiment 2: mortality (mean ± SE) of *Rhagoletis indifferens* exposed to 10 drops of 50 µl of 0.0092% spinosad alone in the presence of sugar only and sugar + yeast extract at 2–48 h after exposure.

Experiment 3: effects of enhanced GF-120 on feeding responses

During 1-h observations, numbers of feeding events among baits differed (Figure 5A), with the highest numbers on GF-120 and GF-120 + UA ($F_{5,30} = 6.2$, P = 0.0004). Mean (±SE) feeding durations per 10 flies on GF-120, GF-120 + UA, and GF-120 + AA (n = 5, 6, and 3 replicates, respectively) were 2.95 ± 0.91, 2.06 ± 0.66, and 2.42 ± 1.82 min, respectively (P>0.05). The duration spent by flies on leaves not feeding (Figure 5B) was higher in the GF-120 treatment than on all other treatments except for GF-120 + UA ($F_{5,30} = 3.1$, P = 0.0215).

Discussion

In experiment 1, several conclusions emerged with respect to mortality of *R. indifferens* in the two food treatments. Overall, results support the hypothesis that the absence of yeast extract results in higher mortality of flies exposed to GF-120, either by itself or when it is enhanced with ammonia compounds. One possible explanation for this is that flies in the absence of yeast extract (sugar only) had a higher drive than flies in the presence of sugar + yeast extract to seek nitrogenous food, and were more active, causing them to encounter the baits relatively quickly and feed. In *Anastrepha ludens* (Loew), access to sugar only increased the feeding preference for yeast hydrolysate (Robacker, 1991). Results with *R. indifferens* in the current study also may be the result of more flies exposed to sugar + yeast extract being 'arrested' near the GF-120 baits and not feeding, than flies exposed to sugar only. Pelz et al.

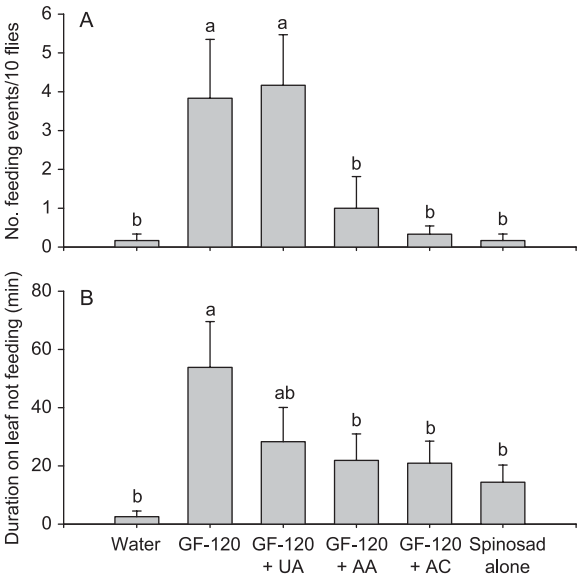

Figure 5 Experiment 3: behavioral responses (mean + SE) of *Rhagoletis indifferens* to fresh enhanced GF-120 in the laboratory between 0–1 h after exposure. (A) Number of feeding events, and (B) duration spent on leaves and not feeding. Sexes were combined. Means capped with same letters are not significantly different ($P>0.05$). UA = 10% uric acid, AA = 10% ammonium acetate, AC = 10% ammonium carbonate.

(2005) used the term 'arrestment' as defined by Kennedy (1978) (which, in part, is a congregation of insects as a result of undirected, kinetic reactions, including the slowing down or stopping of locomotion) to describe behaviors of blueberry maggot, *Rhagoletis mendax* Curran (Pelz et al., 2005), and *R. cingulata* (Pelz-Stelinski et al., 2006) near GF-120. Another possibility is that in the presence of sugar + yeast extract, *R. indifferens* flies avoided or were repelled by the bait, and were not arrested. The addition of ammonia compounds to GF-120 did not influence the effects of the presence of sugar + yeast extract on mortality. This indicates that GF-120 + added ammonia did not cause flies exposed to sugar + yeast extract to feed more than on GF-120 alone over 48 h, and suggests there would be little benefit in adding ammonia to GF-120 for control of *R. indifferens*.

In experiment 1, in contrast to the results with GF-120 baits, mortalities of flies caused by spinosad alone were not affected by the absence or presence of sugar + yeast extract. Thus, in the presence of spinosad alone, feeding on sugar + yeast extract did not appear to reduce the movement of flies any more than feeding on sugar only, resulting in flies contacting the spinosad or ingesting it. It is assumed that flies could not detect and avoid the spinosad. If so, flies may have suffered higher mortalities when exposed to GF-120 baits in the presence of sugar only than in the presence of sugar + yeast extract, because sugar + yeast extract feeding and exposure to bait odors combined caused arrestment or avoidance behaviors.

The hypothesis that mortality caused by spinosad alone is less than that caused by baits with spinosad was not supported. This suggests that bait was not needed for flies to contact spinosad over the 48 h. Spinosad alone apparently was fed upon or contacted as often as the GF-120 baits. There is some evidence that this may also occur in the field. Spinosad alone sprayed on cherry and apple trees did not differ from GF-120 in reducing larval infestations of *R. indifferens* and *R. pomonella*, respectively (Yee, 2007a,b), and behavioral responses to spinosad and GF-120 did not differ (Yee, 2007a).

In experiment 2, the presence of sugar + yeast extract reduced mortalities of *R. indifferens* when it was presented with GF-120, Mazoferm, and Baker's YE, but not with Nu-Lure and spinosad alone, supporting the hypothesis that fly mortality is dependent on the type of bait. Neither Nu-Lure nor spinosad alone in the presence of sugar + yeast extract seemed to cause arrestment or avoidance, and flies may have readily walked onto or fed on the toxin, accounting for the high mortalities. It is unlikely that flies preferred Nu-Lure over the sugar + yeast extract, because *R. indifferens* did not respond to it any more than to water (Yee, 2007a).

In experiment 3, behavioral responses provided a few clues to the mechanism of kill that may explain mortality patterns in experiment 1. Ingestion of bait rather than random body (i.e., tarsal) contact with baits was the main mechanism of kill. In addition, flies fed frequently on GF-120 and GF-120 + UA, but less frequently on GF-120 + AA or AC, which did not follow expectations from experiment 1. This suggests that in experiment 1, GF-120 + AA or AC may have been initially repellent, because they emitted too much ammonia during the 60 min of exposure, and that most flies ingested baits after the ammonia emission decreased to levels that were not repellent. In experiment 3, the relatively long duration spent by flies near GF-120 without feeding may have been a result of flies resting near GF-120 on leaves after feeding, although arrestment cannot be ruled out. Further experiments that include foods and entail observations over 48-h periods are needed to confirm interpretations.

In summary, overall results suggest that the indirect effects of yeast extract food on mortality are dependent on bait type and that mortalities caused by spinosad alone and baits are similar. Nu-Lure and spinosad alone may have an advantage over other treatments for control of *R. indifferens*, because their effects do not appear to be affected by the presence of nitrogenous food. However, future studies should examine the effects of feeding on cherry juice

and bird feces, which are natural foods of *R. indifferens* (Yee, 2008), on the responses of flies to baits in the field, to determine if they have similar effects as feeding on sugar and sugar + yeast extract under laboratory conditions.

Acknowledgements

I thank Katie Mackie, Janine Jewett, and Julie Schlaman (USDA-ARS) for assistance; Joe Morse (University of California, Riverside), Steve Garczynski (USDA-ARS), and Peter Landolt (USDA-ARS) for helpful comments on the manuscript; and Dave Horton (USDA-ARS) for help with statistics. This study was partially funded by the Oregon Sweet Cherry and Washington Tree Fruit Research Commissions. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

References

Alston D & Lindstrom T (2006) Western cherry fruit fly control: can addition of adult attractants enhance the efficacy of GF-120? Available at http://utahpests.usu.edu/ipm/files/uploads/PDFDocs/res06-wcff_evaluation.pdf.

De Deken R, Speybroeck N, Gillain G, Sigue H, Batawi K & Van Den Bossche P (2004) The macrocyclic lactone 'spinosad', a promising insecticide for tsetse fly control. Journal of Medical Entomology 41: 813–818.

Drew RAI, Courtice AC & Teakle DS (1983) Bacteria as a natural source of food for adult fruit flies (Diptera: Tephritidae). Oecologia 60: 279–284.

Huang F & Subramanyam B (2007) Effectiveness of spinosad against seven major stored-grain insects on corn. Insect Science 14: 225–230.

Kennedy JS (1978) The concepts of olfactory 'arrestment' and 'attraction'. Physiological Entomology 3: 91–98.

Littell RC, Milliken GA, Stroup WW & Wolfinger RD (1996) SAS System for Mixed Models. SAS Institute, Cary, NC, USA.

Mangan RL, Moreno DS & Thompson GD (2006) Bait dilution, spinosad concentration, and efficacy of GF-120 based fruit fly sprays. Crop Protection 25: 125–133.

McQuate GT, Sylva CD & Jang EB (2005) Mediterranean fruit fly (Dipt., Tephritidae) suppression in persimmon through bait sprays in adjacent coffee plantings. Journal of Applied Entomology 129: 110–117.

Pelz KS, Isaacs R, Wise JC & Gut LJ (2005) Protection of fruit against infestation by apple maggot and blueberry maggot (Diptera: Tephritidae) using compounds containing spinosad. Journal of Economic Entomology 98: 432–437.

Pelz-Stelinski KS, Gut LJ & Isaacs R (2006) Behavioral responses of *Rhagoletis cingulata* (Diptera: Tephritidae) to GF-120 insecticidal bait enhanced with ammonium acetate. Journal of Economic Entomology 99: 1316–1320.

Prokopy RJ, Cooley SS, Galarza L, Bergweiler C & Lauzon C (1993) Bird droppings compete with bait sprays for *Rhagoletis pomonella* (Walsh) flies (Diptera: Tephritidae). Canadian Entomologist 125: 413–422.

Prokopy RJ, Miller NW, Piñero JC, Barry JD, Tran LC et al. (2003) Effectiveness of GF-120 fruit fly bait spray applied to border area plants for control of melon flies (Diptera: Tephritidae). Journal of Economic Entomology 96: 1485–1493.

Prokopy RJ, Papaj DR, Hendrichs J & Wong TTY (1992) Behavioral responses of *Ceratitis capitata* flies to bait spray droplets and natural food. Entomologia Experimentalis et Applicata 64: 247–257.

Robacker CC (1991) Specific hunger in *Anastrepha ludens* (Diptera: Tephritidae): effects on attractiveness of proteinaceous and fruit-derived lures. Environmental Entomology 20: 1680–1686.

SAS Institute Inc (2004) SAS/STAT® 9.1 User's Guide. SAS Institute, Cary, NC, USA.

Thomas DB & Mangan RL (2005) Nontarget impact of spinosad GF-120 bait sprays for control of the Mexican fruit fly (Diptera: Tepritidae) in Texas. Journal of Economic Entomology 98: 1950–1956.

Vargas RI, Miller NW & Prokopy RJ (2002) Attraction and feeding responses of Mediterranean fruit fly and a natural enemy to protein baits laced with two novel toxins, phloxine B and spinosad. Entomologia Experimentalis et Applicata 102: 273–282.

Webster RP, Stoffolano JG & Prokopy RJ (1979) Long-term intake of protein and sucrose in relation to reproductive behavior of wild and laboratory cultured *Rhagoletis pomonella*. Annals of the Entomological Society of America 72: 41–46.

Yee WL (2006) Feeding history effects on feeding responses of *Rhagoletis indifferens* (Dipt., Tephritidae) to GF-120 and Nulure. Journal of Applied Entomology 130: 538–550.

Yee WL (2007a) GF-120, Nu-Lure, and Mazoferm effects on feeding responses and infestations of western cherry fruit fly (Diptera: Tephritidae). Journal of Agricultural and Urban Entomology 23: 125–140.

Yee WL (2007b) Attraction, feeding, and control of *Rhagoletis pomonella* (Diptera: Tephritidae) with GF-120 with added ammonia in Washington state. Florida Entomologist 90: 335–342.

Yee WL (2008) Feeding substrates and behaviors of western cherry fruit fly (Diptera: Tephritidae). Environmental Entomology 37: 172–180.

Yee WL & Chapman PS (2005) Effects of GF-120 concentrations on attraction, feeding, mortality, and control of *Rhagoletis indifferens* (Diptera: Tephritidae). Journal of Economic Entomology 98: 1654–1663.